

# FATE AND HONEY BEE TOXICITY OF SELECTED PUBLIC HEALTH INSECTICIDES

A Thesis

by

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## ABSTRACT

Honey bees (*Apis mellifera*) are arguably the most important beneficial insect in agricultural and horticultural ecosystems in the United States. Despite their importance, honey bee populations have been negatively impacted by a number of factors, including disease, pests, and exposure to pesticides. Some of these pesticides are used to control mosquitoes, which are both a nuisance and a vector of human and animal diseases. Therefore, public health districts have been established around the U.S. to control mosquito populations through the controlled application of insecticides. Spray drift is a common concern when applying pesticides, particularly with regard to deposits onto highly sensitive unintended targets, such as waterways and honey bee hives. Drift modeling is available for most sprayer models and application techniques, but the truck-mounted spray systems used in public health insect control have much fewer options.

The fate and risk of commonly-used public health insecticides in waterways was evaluated in a literature review. Not nearly enough is known about these pesticides and their chronic and sub-lethal effects or their potential combined effects. Urban runoff is often much more contaminated than agricultural runoff due to lack of monitoring and higher application rates per unit area.

The impact of commonly-used public health insecticides used for mosquito control on honey bee mortality was assessed. The insecticides tested had negligible impact on honey bee mortality even at maximum usage rates. Applicators should still use these products with caution when making public-health applications in areas around apiaries because chronic and sub-lethal effects were not tested.

AGDISP<sup>®</sup> with inputs that emulated a field study setup was used to determine drift from a public health insecticide sprayer. Once the model was validated for this kind of application, the model and the inputs were then used to determine depositions and concentrations at various distances from the spray swath. The deposition and concentration values were then compared to available honey bee and aquatic toxicity values for the specific products in question. These comparisons showed that in these conditions, buffer zones were not needed for these products.

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## CHAPTER I

### INTRODUCTION

Application of public health insecticides for the control of mosquito populations has increased dramatically with the introduction of West Nile Virus and its increasing incidence and geographic dispersion, leading to the founding of permanent mosquito control districts in many states (Schleier et al. 2008). Many of the pesticides used are pyrethroids aided by the synergist piperonyl butoxide (PBO) and are generally regarded as safe for use near mammals (Schleier et al. 2008). Their effects on non-target invertebrates are not as well documented, and they are highly toxic to some aquatic organisms (Gan et al. 2005). The honey bee, *Apis mellifera*, is one of the non-target invertebrate species that is at risk of exposure to pesticides used to control insects that vector pathogens to humans and animals (Caron 1979, Pankiw and Jay 1992). Exposure to pesticides has been long suspected to be a potential cause of declines in the population of the honey bee (Faucon et al. 2002, Finley et al. 1996, Mullin et al. 2010, Johnson 2015), and with the appearance of Colony Collapse Disorder (CCD) pesticides have been under more scrutiny.

Few pesticides have been extensively studied (Gilliom 2001), and there is still much more to learn about how these compounds act in various natural systems. Out of the hundreds of compounds registered for use in the United States, many do not have established standards for drinking water and fewer have guidelines for aquatic life (Gilliom 2001). Those that do have aquatic life standards are based on exposure over only a few days, not chronic exposure or seasonal periods of high concentrations and organisms are often exposed to mixtures of various contaminants but only individual compounds have guidelines (Gilliom 2001). Chapter II is a review of the literature covering the fate, transport, and risk of these insecticides.

Many insecticides are toxic to honey bees in varying doses (Halm et al. 2006; Decourtye et al. 2004; Desneux et al. 2007). Furthermore, Womeldorf et al. (1974) found that concentrated applications of fenthion and dichlovos directly on honey bees caused high mortality rates; however, the effects were not as drastic when using field colonies because the chemicals did not persist in the environment long enough to enter the hives and expose the entire colony. The authors commented that applying either fenthion or dichlovos on actively foraging bees would

never be wise, as this would result in high mortality for those colonies. Atkins et al. (1981) had similar results with bendiocarb, malathion, and chlorpyrifos and found that, while these chemicals were deadly to bees when applied directly on the bees, the mortality rate was negligible when the bees were in their hive at the time of spraying. Atkins et al. (1981) also found that pyrethrins (final concentration of 2.5% and applied at a rate of 2 fl oz/min) had very little effect when directly applied to caged honey bees. These findings implied that precautions should be taken to ensure that honey bees are not foraging or clustering outside hives before spraying agrochemicals near apiaries.

Chapter III assesses the impact on honey bee mortality of commonly-used public health insecticides used for mosquito control. Honey bees were placed in a bioassay cage that was then inserted into a wind tunnel. Through a series of serial dilutions and subsequent exposure of honey bee workers to five products, the expected mortality of honey bees exposed to these insecticides was determined after 24 hours, and the lethal concentrations (LC) values that resulted in 10 and 50% mortality ( $LC_{10}$  and  $LC_{50}$ , respectively) were determined.

Buffer zones, areas that set a minimum distance from a pesticide application swath to a sensitive, non-target area to limit the impact of drifting pesticide (Payne et al. 1988), are common near water bodies but not nearly as common around honey bee hives. Among the many variables that affect the drift of applied chemicals, Hewitt (2000) found that across many studies, the most important factors affecting drift were droplet size, boom height, and wind speed.

Several related studies have been done covering drift, environmental concentrations of insecticides, and buffer zones. Payne et al. (1988) of the Canadian Forestry Service (CFS) came up with a technique for estimating buffer zones using permethrin, a synthetic pyrethroid. The procedure used a worst-case scenario to determine the suggested buffer. This approach, however, did not reflect common application practices and resulted in a larger buffer than was necessary for the majority of spray conditions. This large buffer, in turn, reduced deposition efficiency for controlling the targeted pests (Payne et al. 1988). La Mer and Hochberg (1948), simply suggested that selecting favorable meteorological conditions and carefully controlling droplet size will give the most control over deposition.



The goal of the research in Chapter IV was to provide recommendations for buffer zones to prevent contamination of adjacent water bodies and honey bee hives through field-measured deposition data and associated AGDISP<sup>®</sup> modeling over a range of distances.

## CHAPTER II

### TRANSPORT, FATE, AND RISK OF SELECTED PUBLIC HEALTH INSECTICIDES IN WATERWAYS

#### 2.1. Introduction

Application of public health insecticides for the control of mosquito populations has increased dramatically with the introduction of West Nile Virus and its increasing incidence and geographic dispersion, leading to the founding of permanent mosquito control districts in many states (Schleier et al. 2008). Many of the pesticides used are pyrethroids aided by the synergist piperonyl butoxide (PBO) and are generally regarded as safe for use near mammals (Schleier et al. 2008). Their effects on non-target invertebrates are not as well documented, and they are highly toxic to some aquatic organisms (Gan et al. 2005). Mosquito control is often controversial because of the potential impacts on health and environment due to the chemicals used.

Few pesticides have been extensively studied (Gilliom 2001), and while most of the ones included in this review have been included in many studies, there is still much more to learn about how these compounds act in various natural systems. Out of the hundreds of compounds registered for use in the United States, many do not have established standards for drinking water and fewer have guidelines for aquatic life (Gilliom 2001). Those that do have aquatic life standards are based on exposure over only a few days, not chronic exposure or seasonal periods of high concentrations and organisms are often exposed to mixtures of various contaminants but only individual compounds have guidelines (Gilliom 2001).

The emphasis in this review is to examine the transport, fate, and potential aquatic effects of the active ingredients in selected public health insecticides used for mosquito control. The specific products being investigated and their active ingredients are Aqua-Pursuit™ (permethrin, PBO), Duet® (prallethrin, sumithrin, and PBO), Fyfanon® (malathion), Scourge® (resmethrin, PBO), and Zenivex® (etofenprox). While permethrin, prallethrin, sumithrin (also called phenothrin), resmethrin, and etofenprox are all pyrethroids, malathion is an organophosphate insecticide. This review will cover all of these compounds with the exception of prallethrin and PBO. As far as

the author is aware, no studies have been done on the fate and transport of prallethrin or its toxicity to aquatic invertebrates. PBO is used as a synergist in many of the products, but it has very low toxicity to invertebrates on its own (Schleier and Peterson 2013, Davis et al. 2007).

## 2.2. Transport, Fate, and Risk

Factors affecting the transportation of pesticides into bodies of water include drift (aerial dispersion by wind), volatilization (Siebers et al. 2003), and runoff from rain or irrigation water. Factors influencing pesticide runoff may include the severity of rainfall, length of time from application of insecticide to rainfall, topography of the land, the physiochemical properties of the pesticide (water solubility, degradation rate, volatility), agronomic methods (pesticide formulation and placement), and various soil characteristics such as infiltration, water content prior to rainfall event, cultivation, and surface crusting (Hill 1989). The length of time these pesticides persist in the environment and their concentrations are the major factors when determining toxicity (Medina et al. 1999). Their occurrence (where they are present, in what concentrations, and for how long) is a function of many different chemical and physical properties, such as pH, temperature, presence of organic material, sediment, or other dissolved compounds, the presence or lack of light, and the presence of microbes (Lartiges and Garrigues 1995, Miles and Takashima 1991, Sharom and Solomon 1981, and Vasquez et al. 2011b). Comparing results from different studies is difficult due to different approaches to data collection and differences in geographic areas, such as quantity and types of pesticide used, time of pesticide application, and differences in climate (Gilliom 2001, and Amweg et al. 2006). Depending on the study, it might be deemed that stormflow conditions (Phillips and Bode 2004), low flow conditions (Sengupta et al. 2013), or immediate post-application (Weston 2004) is the best time for sample collection. However, the U.S. Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) program found that concentrations of pesticides followed seasonal patterns that reflected use and hydrologic conditions (Gilliom 2001). This implies that both seasonal patterns of pesticide use and hydrologic patterns in a geographic area (including irrigation and rainfall patterns) should be considered when sampling.

### 2.2.1 Transport into Waterways

Hewitt (2000) has summarized the findings from several spray drift studies by the Spray Drift Task Force (SDTF), which is composed of several companies with registered pesticides with the goal to conduct studies jointly to provide data on spray drift and droplet size for pesticides. The studies conducted by the SDTF ranged from aerial and ground rig field studies to modeling spray drift and atomization using AgDRIFT<sup>®</sup> and DropKick<sup>®</sup>, respectively. The SDTF studies found the most important factors affecting drift to be spray quality, boom height, and wind speed. Hewitt also cites AGDISP<sup>®</sup> as one of the useful models for predicting spray drift. Modeling and predicting drift can help applicators understand what impact meteorological conditions and application techniques have on drift. These studies, though, did not give in-depth discussions on how to reduce spray drift or study drift reduction options.

In the case of volatilization as a transport mechanism, Siebers et al. (2003) took selected active ingredients chosen for their physical and chemical properties. They then determined the concentrations found in air and surface water after treating nearby fields using a range of distances and weather conditions to emulate transport of these pesticides over short ranges. They determined that volatilization and the deposition from it should, in fact, be considered when looking at the entry of volatile pesticides into waterways.

### 2.2.2 Factors Affecting Occurrence and Toxicity

All of the pesticides examined in this review strongly sorb to sediment and soil (Miles and Takashima 1991, Sharom and Solomon 1981, and Vasquez et al. 2010), and fine-grained sediments of silt and clay have a greater capacity for binding contaminants than the coarse grains of sandy soils (Key et al. 2011). This decreases the risk of groundwater contamination while increasing the risk of surface water and sediment contamination through runoff (Miles and Takashima 1991). While binding to sediment prevents bioavailability and limits detrimental effects to many non-target organisms, it also limits the bioavailability of the compounds to be broken down and can lead to build up of a contaminant (DeLorenzo and De Leon 2010). Pesticide occurrence in ground water is most frequent where groundwater is shallow and pesticide use is highest, though generally groundwater is more protected from pesticide

contaminants (Gilliom 2001). Studies by the NAWQA program found that only about 25% of groundwater samples contained 2 or more pesticides, compared to the occurrence of pesticides in streams where 96% of agricultural streams and 99% of urban streams and 70% of the stream water samples tested had five or more compounds (Gilliom 2001).

While knowing the physiochemical properties of a pesticide enables predictions of its fate (Vasquez et al. 2010), it is also important to know the properties of the soil and waterbodies near the application area in question and what other compounds may be present to know how a compound will react and if it will degrade (Pehkonen and Zhang 2002, Lartiges and Garrigues 1995, and Druzina and Stegu 2007). According to the Canadian Forestry Service, when predicting toxicity and the toxicological effects of insecticides in water bodies, many factors must be considered, including the amount of insecticide, its formulation, organism sensitivity and behavior, water flow and rainfall, pH, ionic content, and suspended solids in the water (Payne et al. 1988).

Plants can also help to remove the pesticides from streams and soil. A review of plant-based remediation of pesticides in soil and water can be found in Karthikeyan et al. (2004). Plants release a number of nutrients into the soil around their root systems (the rhizosphere), making these areas primary spots for microorganism growth, which can aid in the degradation of pesticides. They also add organic matter to soil, which can increase the transformation rate of pesticides. Aside from the soil around plants being better for the degradation of pesticides, the plants themselves can help by drawing in pesticides with their water intake, adsorption from soil water to roots, and adsorption from air onto plant stems and leaves. Once within the plant, they may be retained or metabolized (Karthikeyan et al. 2004). Retaining the pesticides can slow the release and decrease the concentrations in soil and waterways, and metabolizing the pesticides can result in less toxic metabolites. Depending on the pesticide, however, the metabolites may be just as toxic or more (Gilliom 2001). This is illustrated in a study of agricultural drainage ditches where Bennett et al (2005) determined that no more than 300 m of vegetated ditch is needed to remove pyrethroids successfully from runoff to levels below detection limits. The highest concentrations of the tested pyrethroids in and on plants were within the first 50 m, and sorbed into soil within the first 25 m. The pyrethroids were below detection limits on both soil and plants before the study was initiated.

Among the many factors affecting the fate and occurrence of pesticides is whether the application is done in agricultural or urban dominated watersheds. Phillips and Bode (2004) studied pesticides in runoff in New York State. They chose to look at pesticide concentrations in stormflow conditions assuming these conditions would result in higher concentrations. They noted that the type of pesticides as well as the application rate and timing is significantly different between developed and agricultural areas. Because of this and the differences in pesticide movement to streams, studies done in agricultural areas have little application in developed areas. It has been suggested that, while the actual values used are unavailable, the higher concentrations in urban areas could potentially be due to increased rates per unit area. They found that no concentrations surpassed any human health-based standards for the compounds that had water quality standards, while some exceeded the standards for aquatic life protection. Half of the compounds found had no such health-based or aquatic-life-based standards, and this is not uncommon, as mentioned by Gilliom (2001). Phillips and Bode (2004) found that mobility was a good predictor of whether the compounds would be detected; those that had very low mobility were not detected very often, whereas those with the largest concentrations were those with higher mobility. However mobility and persistence did not explain all of the results, indicating that other factors had effects as well. This is illustrated most simply by LeBlanc and Kuivila (2008), who found that while suspended sediment and bed sediment concentrations did not show consistent trends, in both water and sediments the pesticides most heavily used were most commonly present. While distribution and occurrence of pesticides in water as well as suspended and bed solids is a complicated function of physical and chemical properties, pesticide use, and watershed residence times, the pesticides most often detected and in the highest concentrations were the most heavily used in the watershed.

Amweg et al. (2006) hoped to determine how widespread pyrethroids are in different urban systems by comparing urban areas in Sacramento, California, and Nashville, Tennessee, and if the concentrations were such that aquatic toxicity would be an issue. Differences in climate, residential development, and pesticide use practices cause differences in the observed toxicity among urban areas. Pyrethroids are used by professional applicators and are the active ingredients in many insecticides available for use by homeowners. They found that 12 out of 15 creeks in California were toxic on at least one occasion and every sample had at least one pyrethroid while most had five of the seven that were tested. Conversely, no creeks in Tennessee

ever tested as toxic. In fact, pyrethroids were rarely detected or were barely above detection levels in the sediments of Nashville creeks. Amweg et al. (2006) also used the toxic unit (TU) approach using *Hyalella azteca*, but this method makes it impossible to determine if other untested substances contribute to sediment toxicity. Permethrin made up 38% of the pyrethroids present in the California samples, but because of the lower aquatic toxicity compared to other pyrethroids, it was not a major contributor to observed mortality.

Jiang et al. (2011) used  $^{14}\text{C}$ -permethrin on concrete to understand better the effects of adsorption and desorption on stream loads in urban settings. In the adsorption experiment, they observed a very rapid uptake of  $^{14}\text{C}$  from the aqueous phase, with equilibrium being reached within 24 hours. The desorption experiment, intended to model the desorption of pesticides to runoff water after a rainfall event or irrigation, showed that 60% of the total  $^{14}\text{C}$ -permethrin desorbed over 300 h happened in the first 24 h, and slowed considerably over time.

Vegetated agricultural ditches appear to prevent pyrethroids from moving very far from their source, but greater flow velocities due to impervious surfaces are likely in urban watersheds. This can result in greater transport of sediment and adsorbed insecticides (Hintzen et al. 2009).

Ding et al. (2010) found that their urban samples had higher concentrations of measured pyrethroids. Differences in concentrations are most likely due to a reduced use of insecticides and greater use of herbicides on the major agricultural crops in Illinois, which are less toxic to benthic organisms, and the financial restrictions of application costs of insecticides on multiple acres of land (using it for profit in a business rather than to take care of pests around a personal lawn). The pyrethroids in this study are all restricted-use in the state of Illinois, meaning applicators employed to use them must have a license, while those applying to their private land do not and may apply the products in greater quantities and more frequently than application procedures dictate. At urban sites, the pyrethroid most frequently detected was bifenthrin, with permethrin being second, but their half-lives are significantly different, ranging from 428 to 483 d and 60 to 312 d for bifenthrin and permethrin, respectively. The differences in half-lives or differences in use could explain the occurrence differences between bifenthrin and permethrin. All of the samples from urban sites contained at least one pyrethroid, while 68% contained two or more. At agricultural sites, the most frequently detected pyrethroid was permethrin, though always at low concentrations. Compared to other urban studies of pyrethroid use, Illinois had

lower concentrations than similar studies in California and Texas, yet higher concentrations than Tennessee (Hintzen et al. 2009, Holmes et al. 2008, Amweg et al. 2006, and Weston et al. 2005). It should be noted that differences in the application time and the amounts, climatic differences which affect the sediment loads, as well as differences in the sample collection times of these different studies would affect the comparison here. The agricultural sites in this study can be compared to the results of Weston (2004), where 32% of total samples and 42% of locations on at least one occasion showed significant mortality to the test species. Differences in pesticide use patterns between states due to differences in crops produced (field corn and soybeans in Illinois for which aesthetic does not matter, fruits and vegetables in California that require large amounts of pyrethroids) are the likely reason for this.

#### 2.2.2.1 Pyrethroids

All the pyrethroids in this review are esters, with the exception of etofenprox, an ether compound (DeLorenzo and De Leon 2010). Permethrin reportedly has the lowest aquatic toxicity of all pyrethroids (Weston et al. 2005), though Vasquez et al. (2010) state that etofenprox is even less lethal than permethrin, when it comes to aquatic species. In general, pyrethroids are highly hydrophobic. Because of this, they sorb onto soil and partition into organic matter (Vasquez et al. 2010). Pyrethroids' strong affinity for the solid phase may lead to offsite transport by sediment movement (Gan et al. 2005 and Schleier and Peterson 2013). In fact, in their study, LeBlanc and Kuivila (2008) found permethrin only in sediment and not at all in detectable aqueous concentrations. Sharom and Solomon (1981) found in their study that permethrin applied to water and sediment, when shaken removed more than 95% of applied permethrin in one minute. Then, after four consecutive 10 mL rinses of water, only 7-9% of the adsorbed permethrin had desorbed. Aquatic plants are likely to take up hydrophobic pesticides due to their large surface areas and their cuticles, which are comprised mostly of lipids (Karthikeyan et al. 2004), making them excellent sinks for pyrethroids.

Many products containing organophosphates have been withdrawn from production for residential use; and in their stead, pyrethroids have increased in use (Weston et al. 2005), even though pyrethroids have high toxicity to many aquatic and benthic organisms. Gan et al. (2005) examined the distribution of pyrethroids (bifenthrin and permethrin) as a function of sediment location and found that concentrations increased with further distance downstream from the



source. This implies that though concentrations may be low at the source, sediment from surface erosion in runoff from storms or irrigation can lead to high concentrations in surface water. This challenges any assumptions that pyrethroids are generally immobile because of their strong adsorption onto soil. Palmquist et al. (2011) found that the rate of sedimentation and the movement of particulates determine transport and fate of pyrethroids because of their affinity for sediments, and while sediment bound pyrethroids accumulate with continuous runoff, major rainfall events can wash away the built up contaminants.

In a study to determine the occurrence of chemicals of emerging concern, Sengupta et al. (2013) assumed low flow conditions would lead to the least amount of dilution, resulting in the worst case concentrations of the observed compounds. This study took place in coastal urban rivers with wastewater effluent in a densely populated area with a semi-arid climate. Though permethrin is among the most widely used and persistent synthetic pyrethroids, it was rarely detected in most water column samples. The researchers in this study did not collect sediment, which they acknowledge would have been useful in determining the role of sedimentation as a removal mechanism for hydrophobic chemicals like pyrethroids. This study illustrates the need to be aware of the physiochemical properties of compounds to predict their fate (Vasquez et al. 2010). Because pyrethroids are strongly hydrophobic, determining the toxicity of suspended and bed sediments is more appropriate. Sediments from moving water, while having lower, less extreme concentrations, can still be toxic to test species (Weston et al. 2004). Weston et al. (2004) found that their data suggest that aquatic pyrethroid concentrations tend to be greatest right after use, rather than after heavy rains. This is feasible through irrigation runoff and drift, as well as the 1-2 month half-lives of pyrethroids in aerobic soils in the area of study (California's central valley), where pyrethroids are applied in the summer and most heavy rains occur in the winter.

Schleier and Peterson (2013) used a maximum application rate of  $7.745 \text{ g ha}^{-1}$ , which is 2-4 times greater than the application rate most mosquito control districts use. Other assumptions yielding a worst case scenario included no buffer, wind prevailing toward the water body, ground sprayer truck traveling along the edge of the water, immediate dispersal of insecticide into the water column, and a static water body. Their results suggest ground-based ultra-low volume (ULV) application will not yield concentrations of bioavailable permethrin above

detectable limits in aquatic systems. They determined that these pesticides most likely have very little effects on aquatic organisms.

Hill (1989) found that modeling data showing any one rainfall event will rarely result in runoff of more than 0.2% of the insecticide applied to the field in the case of pyrethroids. In most cases, the entry of pyrethroids is periodic and into small areas, so if the water is flowing, they are quickly diluted and adsorbed to reduce bioavailability. This results in very short exposure times for non-benthic organisms. Hill concludes that spray drift and runoff of pyrethroids are not hazardous to aquatic systems, though more sensitive macroinvertebrates especially near the shoreline might show effects, though the effects should be localized and transient.

Similarly, Frank et al. (1989) found that when using a permethrin formulation with a similar density to water near a slowly moving water body, it forms a surface film, which minimizes the exposure to fish and bottom sediments. They did not examine contaminated sediment running off. However, in both low and high velocity creeks, permethrin residues were low and disappeared in 0 to 6 h after deposition. They concluded that not even sublethal effects would be expected with these residue levels, though the wind never did blow directly toward the creeks in question.

While Hill (1989), Frank (1989), and Schleier and Peterson (2013) have concluded that spray application is unlikely to cause toxic concentrations in waterways, it is important to remember that there are significant differences between professional use of these products and home owner use (Amweg et al. 2006). In professional applications, financial considerations and restrictions based on licensed applicators limit the amount per unit area that is applied (Phillips and Bode 2004), whereas private homeowner use can be significantly increased in urban and suburban areas (Ding et al. 2010).

Hintzen et al. (2009) conducted studies in residential watersheds with the hope of quantifying the occurrence of pyrethroids and other hydrophobic compounds in sediments, as well as determining the sediment toxicity in relation to *H. azteca*. They conducted this study on urban streams in central Texas and noticed no seasonal differences. This may be due to the climate and application throughout the year. They took 50 sediment samples, each containing at least one insecticide, and analyzed them looking for 12 insecticides and degradation products. Hintzen et

al. used a TU approach when determining toxicity. While all samples had at least one insecticide, only 66% of the samples contained a minimum of one TU. Their data indicated that the TU approach was a good indicator of toxicity, with significant mortality rates in 46% of the samples. Any sites where toxicity was higher than the TU values predicted could be explained by the presence of unanalyzed factors. This method of toxicity testing gives rise to several uncertainties. The first is that other contaminants with similar occurrence patterns are potentially responsible. Another uncertainty is inherent to the calculation of TU values.  $LC_{50}$  values are used to calculate TU values, and there can be a significant amount of interlaboratory variability in the determination of  $LC_{50}$  values. This study was not intended to determine the pervasiveness of these compounds, how far they might travel downstream, or the correlation of watershed characteristics and insecticide contamination.

Weston et al. (2005) chose a residential area near Sacramento, CA, with no industry, very little commercial development, and very little agriculture, to limit pesticide sources to residential application. During summer in this area, the primary source of water in streams would be runoff from over-irrigation. Through using the TU method, they found extensive pyrethroid contamination which was determined to be responsible for high toxicity to *H. azteca*. Contrary to the findings of Gan et al. (2005) where concentrations increased with increasing distance, sediment contamination was found to be localized near storm drain outfalls and tributary entrances to main creeks, and outfalls could affect sediment quality 10s to 100s of meters downstream. This results in small contaminated reaches that build up significantly with many outfalls. They also found that when using toxic units to express concentration, nearly all of their sites would be expected to be acutely toxic to *H. azteca*, and, in fact, while *H. azteca* is naturally found in this area in these streams, its occurrence was inversely correlated with pyrethroid TUs. Permethrin was commonly found to have the highest concentration of all the pyrethroids, but, because of its low toxicity to *H. Azteca*, it contributed little to TUs.

The differences between Gan (2005) and Weston (2005) could be explained by different soil characteristics in the areas of study. Soil characteristics have a large impact on whether or not irrigation runoff dominates stream flow, meaning soil characteristics possibly have an impact on concentrations of pyrethroids found in nearby streams (Domagalski et al. 2010). They found that streams with greater discharge transport fine-grained sediment farther downstream, leaving less accumulated pyrethroids. While pyrethroids were detected during winter storms (due to runoff)

in suspended sediments in all tributaries and the San Joaquin River, they were undetected or had very low detection in sandy sediments in the San Joaquin River. The pyrethroids found in the river and tributaries within the San Joaquin Basin are due to agricultural use and structural pest control in urban settings.

Holmes et al. (2008) also used a TU approach to determine the occurrence of pyrethroids in urban waterways and determine the role of pyrethroids in toxic sediments. They found that organic carbon content, grain size of sediments, and temperature all affect the bioavailability, and therefore the toxicity of pyrethroids. Pyrethroids have a tendency to be more toxic at lower temperatures due to slower metabolic rates. As long as the organic carbon content has been broken down, it can decrease the bioavailability of pyrethroids, as Holmes et al. (2008) found that in locations with intact reeds and leaves making up most of the carbon source, total carbon had little effect on bioavailability. Palmquist et al. (2011) found that dissolved organic carbon and dissolved organic matter (DOM) highly influence bioavailability of pyrethroids, and the type and quality of the organic carbon is important. Any discrepancies between modeled and observed toxicities can be explained as the result of differences in bioavailability.

Coarse sediments tended to have less pyrethroid contamination, but the fate of the pyrethroids in these conditions have not been studied in depth. As mentioned previously, pyrethroids don't bind as well to coarser sediments (Key et al. 2011).

Using TU values in a study of pesticides in urban and agricultural waterways in Illinois, USA, Ding et al. (2010) found that while testing for pyrethroids and other pesticides like organochlorine pesticides, pyrethroids were still the major contributors to the TU values. They found that the urban sediment samples were more toxic than agricultural, and that agricultural sediments actually had negligible mortality of *H. azteca*. Insecticide concentrations and composition differences between agricultural and urban areas likely caused these differences.

Like other pyrethroids, etofenprox has low water solubility and a high affinity for soil and sediment. DeLorenzo and De Leon (2010) tested the toxicity of etofenprox on the estuarine species the grass shrimp (*Palaemonetes pugio*). Grass shrimp are comparable in sensitivity to *Daphnia magna*, the standard freshwater invertebrate toxicity test species, making it a good estuarine test species (DeLorenzo and De Leon 2010). Aqueous exposure with sediment was

used for adults and larvae, while only aqueous was used for embryos. Larval stage was found to be the most sensitive, while embryos were the least due to a hard embryonic coat. Grass shrimp consume DOM that has been sorbed to sediment; so if etofenprox is bound to sediment they consume, the shrimp would be exposed orally as well as through the water column, though high binding affinity to sediment may decrease its bioavailability. Presence of sediment was found to decrease the toxicity of etofenprox to grass shrimp in adult and larval stages. Permethrin and resmethrin follow these trends (Key et al. 2005, DeLorenzo et al. 2006). In the case of phenothrin, however, the larval stage was not significantly more susceptible than the adult grass shrimp, except in the presence of sediment. Embryos still had the expected low toxicity (Key et al. 2011). Table 2.1 shows comparative toxicity values for *D. magna* and grass shrimp for each of the pyrethroids.

Table 2.1. Toxicity of pyrethroids on aquatic organisms.

Product	LC50 value (ug/L)	Species	Reference
Permethrin	0.05	grass shrimp larvae	DeLorenzo et al. 2006
	0.21	adult grass shrimp	DeLorenzo et al. 2006
	0.11	<i>D. magna</i>	Davis et al. 2007
Phenothrin (Sumithrin)	0.154	grass shrimp larvae	Key et al. 2011
	0.161	adult grass shrimp	Key et al. 2011
	30000000	<i>D. magna</i>	Davis et al. 2007
Resmethrin	0.35	grass shrimp larvae	Key et al. 2005
	0.53	adult grass shrimp	Key et al. 2005
	0.22	<i>D. magna</i>	Davis et al. 2007
Etofenprox	0.89	grass shrimp larvae	DeLorenzo and De Leon 2010
	1.26	adult grass shrimp	DeLorenzo and De Leon 2010
	0.57	<i>D. magna</i>	DeLorenzo and De Leon 2010

Environmental fate and toxicity of pyrethroids in water bodies are affected by several different factors specific to the water body and the compound. A few of these include temperature, dissolved organic carbon content, organic carbon content in sediment, grain size of sediment,

adsorption affinity of specific compounds to sediment, and microbial presence and activity (Palmquist et al. 2011).

House et al. (2000) explored the role of sediments in permethrin mobility. Permethrin degrades slower in sediments than in overlying water, most likely due to decreased bioavailability to microorganisms (Sharom and Solomon 1981) and has a tendency to be more persistent in sediments of low pH. Mass balance calculations suggested that permethrin is adsorbed into sediment during transport. They found that retention times for permethrin ranged from 4-26 d in suspended sediment and 103-125 d in river bed sediments. House et al. (2002) stated that retention times for permethrin in the river water samples was lower than that of suspended sediment, but no specific numbers were given. However, Schleier and Peterson (2013) state that pyrethroids have half-lives of less than one day in the water column. Because of this, while benthic invertebrates may be at risk, invertebrates with no benthic part to their life cycle will most likely not experience effects from even chronic exposure to pyrethroids (Schleier and Peterson 2013).

As stated before, volatilization should not be overlooked as a potential pathway into waterbodies (Siebers et al. 2003). For etofenprox, Vasquez et al. (2010) determined the Henry's law constant (H) to estimate volatilization from water, the soil-water distribution coefficient (the ratio of concentrations in those phases), and the organic carbon-normalized distribution coefficient under rice-field conditions to determine its partitioning. They found that H for etofenprox was quite low making volatilization a very minor pathway. They also found that it was more likely to sorb to glass and soil than to volatilize.

Allan et al. (2005) conducted experiments with and without bed sediment in both light and dark conditions to examine diffusion of permethrin in bed sediment. The experiment with bed sediment in light conditions developed a thin surface biofilm. Concentrations reached  $1 \mu\text{g L}^{-1}$  in dark and  $0.1 \mu\text{g L}^{-1}$  in light, which suggests that the biofilm provides more opportunity for sorption. The highest concentrations were found in the top of the sediment, sharply decreasing with depth.

Because pyrethroids are so sorptive, they do not move to subsurface soil layers. With or without moisture, pyrethroids remain in what is known as the photolytic zone that is only down to 0.5

mm below the soil surface (Vasquez et al. 2011b). This is the depth up to which photolysis is thought to occur. However, permethrin was detected in pore water down to a depth of 30 mm by Allan et al. (2005). In flooded conditions, light penetration may decrease, but a thin layer of water can aid in indirect photo-induced chemical degradation by increasing the number of reactive species, such as DOM, dissolved oxygen, and nitrates. Because of this, flooded systems are much more complex than air-dried systems. Vasquez et al. (2011b) examined the photodegradation of etofenprox, and found that on flooded soil, degradation happened faster (half-life of 3.0 d) than on air dried surface (half-life of 18 d). They compared these results to dark controls and found significant differences indicating photolysis as the main method of degradation. Resmethrin also readily undergoes photodegradation with a half-life of 22 to 47 minutes (Rand 2002).

Vasquez et al. (2011a) found that when anaerobic and aerobic degradation of etofenprox were compared, overall aerobic had a faster rate (half-life of 27.5 d) than anaerobic (half-life of 100 d). Using sterilized controls was significantly different than biologically active soils, implying that microbes play a role in the degradation of etofenprox, though Gan et al. (2005) found that pyrethroids had moderate-to-long persistence in sediment under both anaerobic and aerobic conditions. When in anaerobic, dark conditions, resmethrin has a half-life of 682 d, but under aerobic dark conditions, the degradation rate increased and the half-life was 30 d. However, sterile water with sunlight yielded a half-life of 22-47 m.

Sharom and Solomon (1981) only detected (dichlorovinyl) dimethylcyclopropanecarboxylic acid (DCVA) as a degradation product in their adsorption/desorption study of permethrin. DCVA is much more water soluble than permethrin and therefore more of it remains in the aqueous phase. Degradation products of resmethrin include chrysanthemic acid, ketoaldehyde, and 5-benzyl-3-furoic acid (BFCA) (Rand 2002).

#### 2.2.2.2 Organophosphates (Malathion)

Malathion is one of the most frequently detected pesticides in streams of urban areas (Gilliom 2001). Phillips and Bode (2004) found that the occurrence of malathion was more frequent in urban than non-urban streams, and unlike agricultural areas, peak concentrations occurred

continuously not just throughout the growing season, but even after, which implies several applications were used throughout the year. LeBlanc and Kuivila (2008) found malathion only in aqueous concentrations (not in suspended or bed sediment) in the Salton Sea Basin of California. Malathion was among the most frequently detected pesticide with the highest aqueous concentrations in samples. It was found to have the highest concentrations in late winter and early spring, and at one point had a concentration of 1,100 ng/L. This is above the 48 h LC<sub>50</sub> value of *D. magna*, which is 1000 ng/L (Cheminova Inc. 2002). It was also one of the few pesticides detected offshore in the lake (LeBlanc and Kuivila 2008). Furthermore, in a study comparing several mosquito control pesticides over several species and determining risk quotients by comparing exposure to toxic endpoints (the acute LC<sub>50</sub> values and the chronic, no observed effect values), Davis et al. (2007) found that only amphipods exposed to organophosphates exceeded U.S. Environmental Protection Agency levels of concern. The only aquatic invertebrates in the study were amphipods and *D. magna*. In another study on the grass shrimp by Key et al. (1998), newly hatched larvae were the most susceptible to malathion, with 18-day-old larvae and then adult grass shrimp following, as shown in Table 2.2.

Table 2.2. LC<sub>50</sub> values of malathion for selected aquatic species.

LC <sub>50</sub> values (ug/L)	Aquatic Species	Reference
9.06	Newly hatched grass shrimp larvae	Key et al. 1998
13.24	18 day old grass shrimp larvae	Key et al. 1998
38.19	adult grass shrimp	Key et al. 1998
1.00	<i>D. magna</i>	Cheminova Inc. 2002

Despite the occurrence of malathion, in a study to determine its fate in wastewater treatment plants, sorption to activated sludge was found to be insignificant (Janeczko et al. 2014). In fact, organophosphates may go through a wastewater treatment plant without any changes (Janeczko et al. 2014). This brings awareness to the stability of these products, and it leads to questioning if natural systems are any better at degrading and removing these compounds.



In a study of the degradation kinetics of organophosphates, Lartiges and Garrigues (1995) found that it is difficult to predict their fate and persistence due to the differences in half-life ranges of each of the tested pesticides. Factors that influence the degradation of these pesticides include temperature, pH, light, particulates in water (such as DOM and sediment for adsorption), other compounds and ions in the water, and the chemical structure of the particular pesticide (Lartiges and Garrigues 1995). Getenga et al. (2000) found organic content in the soil also to be an important factor in the fate of malathion, as it absorbs the pesticide and keeps it from leaching. Lartiges and Garrigues (1995) found increased temperature and increased pH generally increase the degradation rate. This was also supported by Druzina and Stegu (2007). For organophosphorus pesticides such as malathion, Medina et al. (1999) found even when microorganisms are present in all samples, the fastest degradation occurred when sunlight, particulates for adsorption, and volatilization were all present as well. Degradation in river, ground, and seawater showed that photolysis and biodegradation were minor pathways, and an elimination reaction controlled degradation (Miles and Takashima 1991), though there is evidence that malathion undergoes direct and indirect photolysis (Remucal 2014).

In a study conducted on the dissipation of  $^{14}\text{C}$ -malathion in soil, Getenga et al. (2000) found that there was a very fast initial decrease in extractable residues of malathion that slowed after the first 20 days after application. Similarly, the bound residues showed an initial increase up until 20 days after application, after which they decreased. Both types of residues decreased faster with more rainfall. The decrease of extractable residues was most likely due to binding to the soil, then leaching with rainfall, vaporization, and biodegradation. This is supported by the rapid degradation observed in the field by Miles and Takashima (1991) with half-lives of two hours.

According to Pehkonen and Zhang (2002), degradation of malathion can also take place due to hydrolysis. While acid catalyzed hydrolysis is too slow to be a major pathway, alkaline hydrolysis is fast enough to be important. While metal catalyzed hydrolysis shows an increased rate, it is unclear if this is an important pathway in natural systems due to a lack of information on the speciation of metal ions (the change in concentration of ions as pH changes) in the presence of common river water components like humic and fulvic acids (Pehkonen and Zhang 2002).

Miles and Takashima (1991) identified degradation products dimethyl phosphorodithioic acid and diethyl fumarate, and Pehkonen and Zhang (2002) reported HCHO (formaldehyde) and dialkyl sulfides as degradation products. The major degradation products depend on which mechanism dominates, and the dominant mechanism depends on the chemical and physical properties of the location (Pehkonen and Zhang 2002).

### 2.3. Summary

It is difficult to summarize the findings of even a single group of pesticides into a simple recommendation, as seen in Druzina and Stegu (2007), Simo and Zhang (2002), and Amweg et al. (2006). This, combined with many potential entry points for pesticides, especially in urban areas where their use is less monitored and they are applied in greater amounts per unit area, leads to the suggestion that a potential focus might be on just how prevalent these pesticides are and what the actual concentrations are in natural systems. Often the concentrations achieved from a single application are not going to cause concerning mortality levels; but multiple, unrelated applications are what cause concern, as seen in Weston (2005). It is also very difficult to determine the sub-lethal chronic effects of exposure to pesticides and the unintended effects of multiple pesticides working together to cause mortality. There's much we do not know yet about the way these compounds act in complicated natural systems, and more studies will need to be performed involving specific compounds and their metabolites and specific areas of concern.

Preventing entrance to waterways should still be the main focus when applying these pesticides. This can be done by applying in prime meteorological conditions in the case of drift and volatilization and preventing runoff by using vegetated buffer zones in agricultural areas and avoiding the application to pavement and hard surfaces in urban areas in the case of runoff.

## CHAPTER III

### TOXICITY OF PUBLIC HEALTH INSECTICIDES TO HONEY BEES

#### 3.1 Introduction

Exposure to pesticides has been long suspected to be a potential cause of declines in the population of the honey bee, *A. mellifera* (Faucon et al. 2002, Finley et al. 1996, Mullin et al. 2010, Johnson 2015). In addition to the potential exposure to pesticides used in agro-ecosystems, honey bees are also at risk of exposure to pesticides used to control insects that vector pathogens to humans and animals (Caron 1979, Pankiw and Jay 1992). To prevent outbreaks of vector-borne diseases, public health insecticide spraying is widespread in the United States. Although the Environmental Protection Agency regulates pesticide use nationwide and requires all pesticides to be registered, individual states and sometimes counties or cities have the authority to establish mosquito control districts. For instance, the Mosquito Abatement District Act of Illinois establishes the legal process of mosquito abatement for “any contiguous territory having a population of not less than 300 inhabitants” forming a district within the state of Illinois (Illinois General Assembly, 2015). Likewise, Division 3 Chapter 1 of the California Health and Safety Code allows for the formation of Mosquito Abatement and Vector Control Districts. Some states, like Delaware and Connecticut, have statewide mosquito control programs within other divisions of the state government.

There are various methods to carry out bioassays to determine the toxicity of insecticides used for mosquito abatement on non-target beneficial insects, including honey bees. One method is to topically apply the pesticide directly on the bee’s thorax (Anderson and Atkins 1968). Another method is to place bees in cages and spray the cages with the pesticides transported in a solvent (Womeldorf et al. 1974, Anderson and Atkins 1968, Atkins et al. 1981). In any of these cage bioassays, the density of the mesh covering the cage in which the test subjects are confined has a significant effect on the results of the bioassays (Hoffmann et al. 2008). Lower mesh densities result in higher mortality or greater effect of the pesticides on the bee mortality. Test cages also introduce a chance for secondary exposure to the chemicals due to direct contact with the cage surface (Bonds et al. 2010). Cage bioassays are simple and repeatable and when making

comparisons between treatments, the technique can be quite useful (Bonds et al. 2010). For instance, in a study involving mosquitos, Hoffmann et al. (2008) tested the efficacy of two types of cages and a wind tunnel, and found that cage mesh density decreased the amount of spray, size of droplets, and wind speed within the cages.

Extensive research on the effects of pesticides on honey bee health has shown that many of these compounds are insecticides commonly used for the control of insect pests that cause damage to agricultural crops and vector several diseases to humans and animals. Many of these insecticides are toxic to honey bees in varying doses (Halm et al. 2006; Decourtye et al. 2004; Desneux et al. 2007). Furthermore, Womeldorf et al. (1974) found that concentrated applications of fenthion and dichlovos directly on honey bees caused high mortality rates; however, the effects were not as drastic when using field colonies because the chemicals did not persist in the environment long enough to enter the hives and expose the entire colony. The authors commented that applying either fenthion or dichlovos on actively foraging bees would never be wise, as this would result in high mortality for those colonies. Atkins et al. (1981) had similar results with bendiocarb, malathion, and chlorpyrifos and found that, while these chemicals were deadly to bees when applied directly on the bees, the mortality rate was negligible when the bees were in their hive at the time of spraying. Atkins et al. (1981) also found that pyrethrins (final concentration of 2.5% and applied at a rate of 2 fl oz/min) had very little effect when directly applied to caged honey bees. These findings implied that precautions should be taken to ensure that honey bees are not foraging or clustering outside hives before spraying agrochemicals near apiaries.

In 1972, blueberry farmers in New Brunswick, Canada were concerned about the lack of pollinators in fields where the insecticide fenitrothion had been sprayed. Kevan (1975) sampled native bee populations for three consecutive days during the time of most pollinator activity and tested dead bees were for the presence of fenitrothion. He examined the exact locations where the insecticide had been sprayed and in what concentrations. He then compared the number of bees collected across all areas near the spray site. Although the author was unable to count the number of bees present at the test sites before sprays were applied, he concluded there had been a decrease in pollinator diversity and abundance as a result of the application of the insecticide.

More recently there has been an increased interest on the effects of neonicotinoid insecticides and their mode of toxicity on honey bees (see Dively et al. 2015 for a review on the topic). For example, Iwasa et al. (2004) made topical applications to the dorsal thorax with various types of neonicotinoid insecticides (imidacloprid, clothianidin, thiamethoxam, dinotefuran, nitenpyram, acetamiprid, and thiacloprid) to determine the toxicity of each chemical to honey bees. They found that topically applied cyano-substituted neonicotinoids had much lower toxicity to bees than nitro-substituted neonicotinoids. The toxicity levels for the seven neonicotinoids tested ranged from LD<sub>50</sub> (the lethal dose that resulted in 50% mortality) values of 18 ng/bee to 14600 ng/bee. Similar studies looking at the toxicity of mosquito abatement insecticides to honey bee foragers is still lacking, however.

The goal of the present study was to assess the impact on honey bee mortality of commonly-used public health insecticides used for mosquito control. Honey bees were placed in a bioassay cage that was then inserted into a wind tunnel. Through a series of serial dilutions and subsequent exposure of honey bee workers to five products, the expected mortality of honey bees exposed to these insecticides was determined after 24 hours, and the lethal concentrations (LC) values that resulted in 10 and 50% mortality (LC<sub>10</sub> and LC<sub>50</sub>, respectively) were determined.

### 3.2 Materials and Methods

#### 3.2.1 Wind Tunnel

A bioassay cage was inserted into a wind tunnel to study honey bee mortality after insecticide exposure. Solutions with varying amounts of insecticide were sprayed through the tunnel. After 24 h, honey bee mortality was assessed to determine lethal concentration (LC) values that resulted in 10% and 50% bee mortality, denoted as LC<sub>10</sub> and LC<sub>50</sub>, respectively.

The wind tunnel used in this study was based on a World Health Organization (WHO) design for evaluating insecticidal activity of active ingredients used as space sprays (WHO 2009). The wind tunnel was constructed from galvanized duct pipe with an internal diameter of 15 cm (Figure 3.1). The entrance of the tunnel was covered with an end cap with ten circular holes with a 2-cm diameter, which regulated the airflow to 3.5 m<sup>3</sup>/s when the motor was adjusted to create airspeed of 2.9 m/s inside the tunnel as specified by WHO (2009). An atomizer with a volume median diameter of 15 ± 2 µm was inserted into the middle of the opening of the tunnel for each spray

treatment to deliver droplets of the test insecticides (Figure 3.1). The droplet size produced by the atomizer caused the carrier (acetone) to evaporate before the active ingredient entered the bioassay cages. Nitrogen was used as the propellant and the pressure was regulated to  $\sim 70$  kPa. For each replication, a bioassay cage with honey bees was placed inside the tunnel, the nitrogen was turned on, and 0.5 mL of the designated spray solution for that test was pipetted into the opening of the nozzle. It took four seconds for an entire solution to be sprayed on a test cage. After waiting for 10 s to ensure that all of the spray had been exhausted from the tunnel, the bioassay cage was removed from the tunnel and another cage was kept in its place for the next replication. Five mL of acetone was sprayed through the nozzle to remove any residual material.

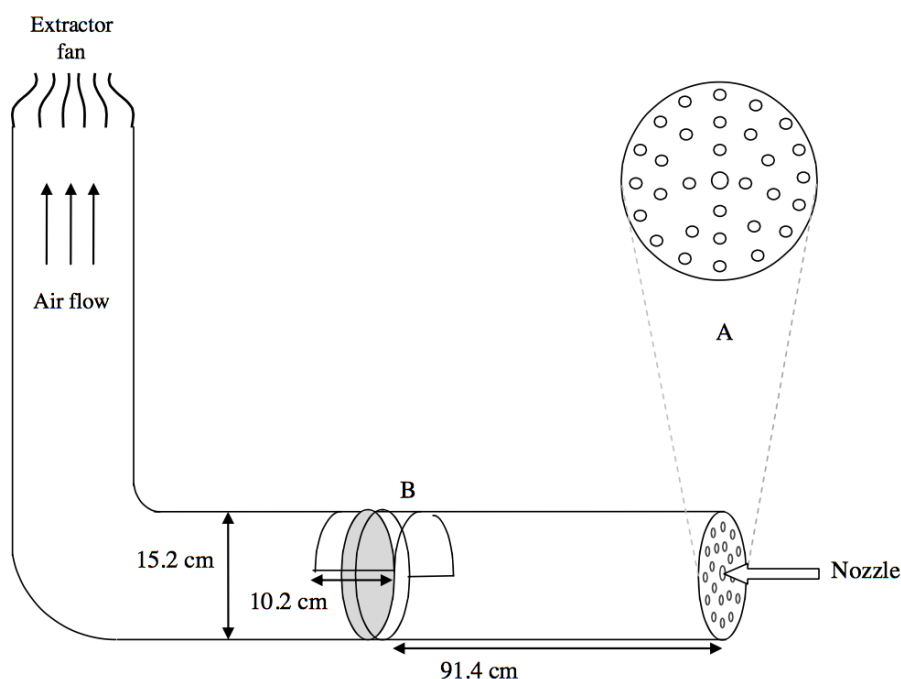


Figure 3.1. Schematic of the WHO Bioassay Wind Tunnel. (Courtesy of WHO 2009).

### 3.2.2 Bioassay Cages

The honey bees used in this study were collected from hives located at the Janice and John G. Thomas Honey Bee Facility of Texas A&M University, in College Station, TX (30.6014° N, 96.3144° W). Prior to the spraying of each treatment-replication combination for each cage bioassay, approximately 25 worker and drone bees were placed in a round brass bioassay cage 15 cm in diameter and 2.5 cm deep. The mesh screen openings on the cage were 1.22 mm × 1.6 mm with a wire thickness of 0.28 mm. This cage was designed at the J. Mulrennan Sr. Public Health Entomology and Research Education Center (PHEREC) in Panama City, FL.

After spraying a cage, the honey bees were transferred to clean, non-sprayed holding cups and were given access to a 50:50 weight by volume sugar syrup feeder. Honey bees used in the control were sprayed with only acetone, then moved to holding cups. The cups were placed in an incubator kept at 34°C and 75% RH. After 24 h, the number of dead honey bees was counted and recorded for each cage. Honey bee mortality was calculated from the observed mortality in the cages (MO) and any mortality observed in the control (MC) using Abbott's corrected mortality equation (Abbott1925).

$$M (\%) = [(MO - MC) / (100 - MC)] \times 100 \quad (3.1)$$

### 3.2.3 Insecticides and Dilutions

Five insecticides routinely used by mosquito control operators for public health spray applications were selected for this study and include:

- Aqua-Pursuit™ (EPA Reg. No. 53883-274-86291, Active Ingredients: 20.6% Permethrin, 20.6 % Piperonyl Butoxide, Precision Control Technology Inc., Baltimore, MD), Maximum labeled rate: 1.7 g permethrin/ha.
- Duet® (EPA Reg. No. 1021-1795-8329, Active Ingredients: 1% Prallethrin, 5% Sumithrin, 5% Piperonyl Butoxide, Clarke Mosquito Control Products, Inc., Roselle, IL), Maximum labeled rate: 0.81 g prallethrin/ha.
- Fyfanon ULV® (EPA Reg. No. 67760, Active Ingredient: 96.5% Malathion, Cheminova Inc., Research Triangle Park, NC), Maximum labeled rate: 67 g malathion/ha.

- Scourge<sup>®</sup> (EPA Reg. No. 432-716, Active Ingredients: 4.1% Resmethrin, 12.4% Piperonyl Butoxide, Bayer Environmental Sciences, Montvale, NJ), Maximum labeled rate: 7.9 g resmethrin/ha.
- Zenivex<sup>®</sup> E20 (EPA Reg. No. 2724-791, Active Ingredient: 20% Etofenprox, Wellmark International, Schaumburg, IL), Maximum labeled rate: 2.0 g etofenprox/ha.

The toxicity effects of these products on honey bees are relatively unknown because each of these products has different active ingredients at different labeled rates, particularly for airborne dosage levels. To determine the initial dosage levels for each product used, a few assumptions were made. First, the initial maximum dosage level for each product was assumed based on the maximum labeled rate (shown above) for that compound. Second, to determine the dilution rate needed for each product, preliminary (unpublished) test data from a previous project the organophosphate insecticide, naled (EPA Reg. No. 5481-481, AMVAC, Los Angeles, CA), were used. A solution of 900 µg/mL of naled diluted into acetone (maximum labeled rate of 112 g/ha) resulted in 100% honey bee mortality, while a 100 µg/mL of naled resulted in nearly 0% mortality. Finally, the targeted maximum and minimum dilution rates for each of the five products were calculated based on equivalence between the maximum targeted rate of the product and that of naled. As an example, the maximum spray rate for Duet<sup>®</sup> was 0.81 g resmethrin/ha compared to 112 g naled/ha. The maximum equivalent dilution for Duet<sup>®</sup> was then calculated as  $(0.81 / 112) \times 900$  to equal 6.5 µg malathion/mL. Similarly, for Aqua-Pursuit<sup>™</sup> the maximum spray rate was 1.7 g permethrin/ha, which resulted in a maximum equivalent dilution rate of 13.7 µg/mL. The maximum equivalent dilutions for Scourge<sup>®</sup>, Zenivex<sup>®</sup> and Fyfanon<sup>®</sup> were similarly determined to be 63.5, 15.8, and 540 µg/mL, respectively. Using the same calculation but substituting 100 for 900 to denote the lowest dilution rate, the minimum equivalent dilutions were determined to be 7.1, 1.5, 0.7, 1.8 and 60 µg/mL for Scourge<sup>®</sup>, Aqua-Pursuit<sup>™</sup>, Duet<sup>®</sup>, Zenivex<sup>®</sup> and Fyfanon<sup>®</sup>, respectively.

Using the determined maximum and minimum equivalent dilution rates for each of the five products, a series of four dilutions were conducted. All dilutions were done with acetone as described by the WHO method (WHO 2009). Acetone was used as a diluent because it evaporates immediately upon spraying, leaving only the product being tested. The dilution ranges were set by targeting as close as possible the maximum and minimum spray rates to those



determined above. Some modification had to be made so that a lower volume requirement could be accurately measured, however. For example, to obtain the required maximum concentration equivalent of 6.5 µg/mL for Duet® an initial stock solution consisting of 150 mL acetone and 0.325 mL Duet® would be required. Further serial dilutions were adjusted to generate a uniform range between the maximum and minimum dilutions established by adjusting both the volume of concentrated solution and acetone used. As an example, the serial dilutions for the initial round of testing using Scourge® are shown in Table 3.1. All dilution calculations were made and recorded using Microsoft Excel. Scourge® contains 0.036 g resmethrin / mL, a concentration that was used to generate a stock solution for subsequent serial dilutions by diluting 5 mL of the formulated product into 100 mL of acetone, resulting in a mixture with 0.0018 g resmethrin / mL. Further diluting 50 mL of this stock solution in 50 mL of acetone results in the first serial dilution containing 900 µg of resmethrin per mL, which is equivalent to 900 parts per billion (ppb). In the table, this is represented as Treatment 5. The serial dilution for Treatment 4 was generated by diluting 50 mL of the first serial dilution solution (Treatment 5) with 50 mL of acetone to make a second serial dilution of 450 µg resmethrin / mL solution. Similarly, Treatment 3 was a dilution of 50 mL of second serial dilution solution (Treatment 4) with 100 mL of acetone to make a 150 µg resmethrin / mL solution. The final (minimum) serial dilution (Treatment 1) was generated through the same process resulting in a solution with 25 µg resmethrin / mL. The spreadsheet allowed for quick determination of serial dilutions to create any desired concentration, which was critical as several additional rounds of testing were required to pinpoint the honey bee mortality levels with several products. The treatments were numbered and performed backwards such that the treatment with the lowest concentration of chemicals was done first in order to minimize contamination between varying dilution levels. This meant that Treatment 1, a control of only acetone, was used as a measure of contamination between products. Subsequent dilution treatments went from lowest active product concentrations to highest.

Table 3.1. Protocol for the serial dilution of Scourge® in acetone to test its effects on honey bee mortality at different concentrations. A.I. is the active ingredient and TRT is the treatment number.

Insecticide: Scourge® (4% Resmethrin (0.3#/gal), 12.4% Piperonyl Butoxide)								
TRT	A.I. Rate lb/gal	A.I. Rate g/mL	Pesticide mL	Acetone mL	Result (g/mL) (g a.i./mL)	Stock (mL)	Acetone (mL)	Final (µg/mL)
5	0.3	0.036	5	100	0.001802	50	50	900
4						50	50	450
3						50	100	150
2						20	100	25
1							100	Control

The first set of trials did not result in mortality levels which contained the LC<sub>50</sub> value (the concentration that killed 50% of the caged honey bees). This required additional test runs with serial dilutions resulting in new maximum and minimum equivalent dilutions for each of the products tested. For Duet®, the second round of testing used dilution rates from 20 to 164 µg prallethrin / mL, which still did not result in treatment levels with 50% mortality. Therefore another set of trials was conducted with dilution rates from 830 to 9000 µg prallethrin / mL. The other three products also required an additional two rounds of testing with adjusted dilution rates. Dilutions with Zenivex® ranged from 27 to 320 µg etofenprox / mL for round two, and from 1350 to 178,000 µg / mL for round three. Dilutions with Aqua-Pursuit™ ranged from 114 to 912 µg permethrin / mL for round two, and from 780 to 200,000 µg/mL for round three. Finally, dilutions with Fyfanon® ranged from 200 to 1130 µg malathion / mL for round two, and from 670 µg to 1.2 g/mL for round three.

### 3.3 Results and Conclusions

To determine the LC<sub>10</sub>, LC<sub>50</sub>, and LC<sub>90</sub> values for each product tested, Morgan-Mercer-Flodin (MMF) sigmoidal models (Eq. 2) (Morgan et al. 1975) were fit to honey bee mortality rates from Abbott's corrected mortality equation (Abbott, 1925) at 24 and 48 h post treatment using CurveExpert (Version 2.0.2; Daniel G. Hyams©). Example plots for the 24 and 48 h mortality data versus dosage levels of Aqua-Pursuit™ are shown in Figures 3.2 and 3.3, respectively.

Using equation 2, the LC<sub>10</sub>, LC<sub>50</sub>, and LC<sub>90</sub> values (i.e., the concentration that killed 10%, 50%, and 90% of the caged honey bees, respectively) for all five products at the 24 and 48 h points were determined (Table 3.2).

$$24 \text{ or } 48 \text{ hour mortality (\%)} = \frac{ab + cx^d}{b + x^d} \quad (3.2)$$

Where:

x = Dosage or concentration of active product applied (µg/mL)

a, b, c, d = constants, determined in CurveExpert when the MMF curve was fit to the data, dependent upon each product and 24 or 48 h mortality data, included in Tables 3.3 and 3.4, respectively.

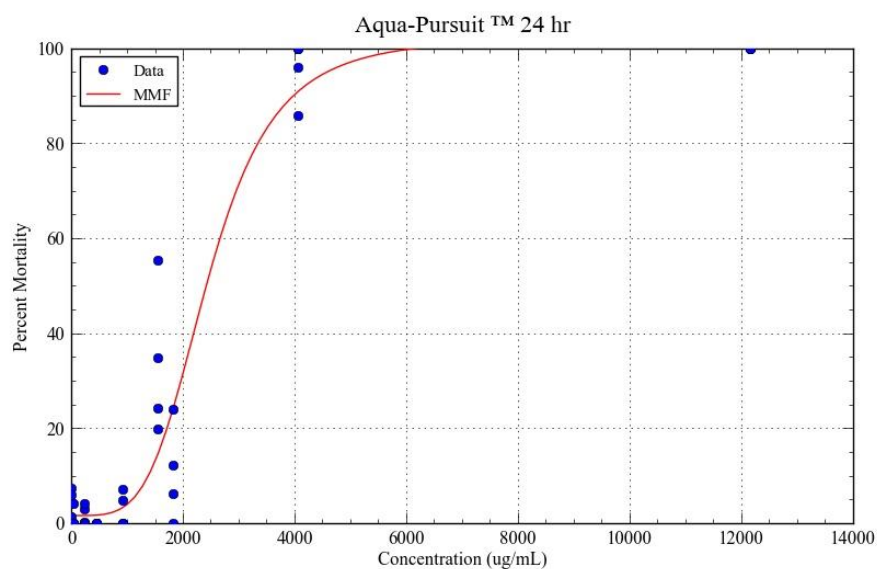


Figure 3.2. Percent honey bee mortality 24 h after treatment with Aqua-Pursuit™ (20.6% Permethrin, 20.6 % Piperonyl Butoxide).

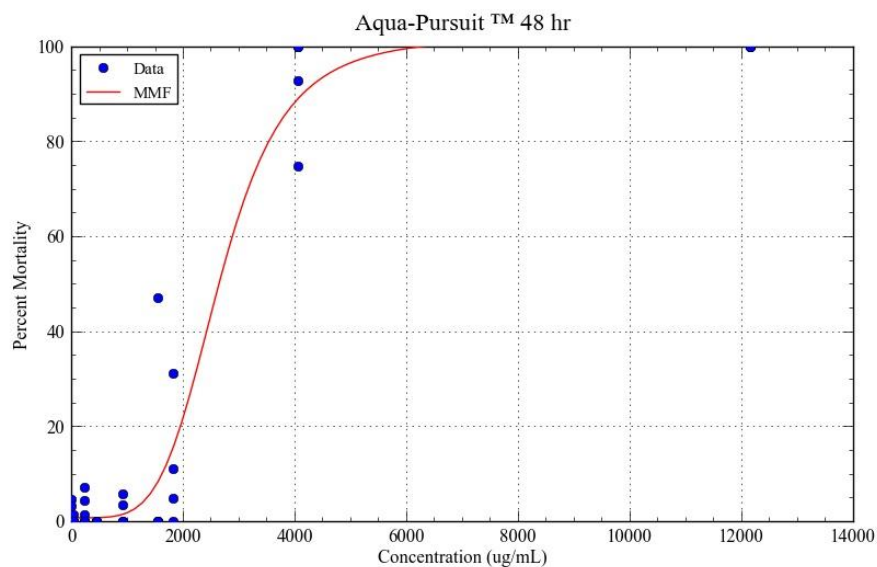


Figure 3.3. Percent honey bee mortality 48 h after treatment with Aqua-Pursuit™ (20.6% Permethrin, 20.6 % Piperonyl Butoxide).

Normal operational usage rates resulted in no significant detrimental health effects on honey bees as a result of exposure to airborne spray concentrations when using the dilution concentrations corresponding to the maximum labeled rate for each product tested. In fact, based on the equivalent maximum labeled rate dilution concentration, the expected mortalities for the products tested would be <2%, near 0%, 11%, near 0%, and <2% for Aqua-Pursuit™, Duet®, Fyfanon®, Scourge®, and Zenivex®, respectively.

Table 3.2. Dilution concentrations corresponding to the LC<sub>10</sub>, LC<sub>50</sub>, and LC<sub>90</sub> values for each product evaluated for the 24 and 48 h honey bee mortality assessment.

Products	Equivalent Max Labeled Rate Dilution Concentration (µg a.i./mL)	24 h			48 h		
		LC <sub>10</sub>	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>10</sub>	LC <sub>50</sub>	LC <sub>90</sub>
Aqua-Pursuit™	13.7	1350	2390	3940	1600	2620	4110
Duet®	6.5	395	2190	4680	380	2150	4620
Fyfanon®	540	516	2430	4960	483	2590	5440
Scourge®	63.5	129	351	607	119	345	607
Zenivex®	15.8	691	1370	2630	687	1380	2670

Table 3.3. 24 h constants used in Equation 3.2.

Chemicals	24 h			
	a	b	c	d
Aqua-Pursuit™	1.82E+00	7.96E+13	1.02E+02	4.10E+00
Duet®	-2.67E+00	6.71E+03	4.23E+02	8.91E-01
Fyfanon®	-4.75E+00	9.28E+03	5.03E+02	9.00E-01
Scourge®	-1.39E+01	3.47E+03	4.56E+02	1.08E+00
Zenivex®	1.43E+00	4.47E+10	1.00E+02	3.39E+00

Table 3.4. 48 h constants used in Equation 3.2.

Chemicals	48 h			
	a	b	c	d
Aqua-Pursuit™	9.26E-01	4.68E+15	1.02E+02	4.58E+00
Duet®	-2.19E+00	6.78E+03	3.95E+02	9.03E-01
Fyfanon®	-3.02E+00	8.93E+03	4.94E+02	8.87E-01
Scourge®	-1.20E+01	3.28E+03	4.81E+02	1.05E+00
Zenivex®	1.73E+00	3.59E+10	1.00E+02	3.36E+00

### 3.4 Summary

The effects on honey bee mortality of commonly-used public health insecticides for mosquito control were assessed in this study. Five insecticides typically used for mosquito control were tested to determine their potential effect on honey bees. Honey bees were exposed to varying airborne concentrations of each insecticide in a wind tunnel and  $LC_{10}$ ,  $LC_{50}$ , and  $LC_{90}$  values were determined for 24 and 48 hours.

It was found that the insecticides tested had negligible impact on honey bee mortality even at maximum usage rates except. The results from this study will provide guidance to mosquito abatement district managers to properly select public health insecticides that cause minimum toxicity effects on honey bees. Our results strengthen the notion that most public health sprays should be done at dusk or later in the day when honey bees are not active outside and remain inside their hives. It should be noted that this study did not examine the potential chronic effects of any of these insecticides at sub-lethal doses.

## CHAPTER IV

### MODELING SPRAY DISPERSION INTO WATERWAYS AND ONTO SENSITIVE AREAS USING A TRUCK-MOUNTED GROUND SPRAYER

#### 4.1 Introduction

Buffer zones, areas that set a minimum distance from a pesticide application swath to a sensitive, non-target area to limit the impact of drifting pesticide (Payne et al. 1988), are common near water bodies but not nearly as common around honey bee (*A. mellifera*) hives. Among the many variables that affect the drift of applied chemicals, Hewitt (2000) found that across many studies, the most important factors affecting drift were droplet size, boom height, and wind speed. Public health spraying has become more widespread in the last few years with the increasing incidence and geographic dispersion of West Nile Virus, causing many states to set up permanent mosquito control districts (Schleier et al. 2008). Many of the pesticides used are pyrethroids and piperonyl butoxide (PBO) and are generally regarded as safe for use near mammals (Schleier et al. 2008). Their effects on non-target invertebrates are not as well documented, and they are highly toxic to some aquatic organisms (Gan et al. 2005).

Colony Collapse Disorder (CCD), the unexplained disappearance of whole honey bee colonies from their hives (Dainat, et al. 2012), has become a growing concern since it first appeared in 2006. Therefore, the fate of the honey bee in relation to pesticide application has become a subject of much scrutiny in recent years. Even pesticides with no visible ties to CCD are being examined more closely for their sub-lethal effects on the honey bee and how they may interact with other factors (Suryanarayanan and Kleinman 2012, Sanchez-Bayo and Goka 2014). Atkins et al. (1981) and Womeldorf et al. (1974) found that direct exposure of fenthion, dichlofos, bendiocarb, malathion, and chlorpyrifos caused high mortality rates in honey bees that have been directly exposed, but otherwise would have little effect on the bees. It stands to reason that applying insecticides to honey bees and their hives, whether directly or through accidental drift, should be avoided.

Several related studies have been done covering drift, environmental concentrations of insecticides, and buffer zones. Payne et al. (1988) of the Canadian Forestry Service (CFS) came up with a technique for estimating buffer zones using permethrin, a synthetic pyrethroid. The procedure used a worst-case scenario to determine the suggested buffer. This approach, however, did not reflect common application practices and resulted in a larger buffer than was necessary for the majority of spray conditions. This large buffer, in turn, reduced deposition efficiency for controlling the targeted pests (Payne et al. 1988). CFS used swath spacing based on operational practices for the equipment and the predominant plant canopy that its organization would be spraying, but otherwise used water to obtain worst-case volatility conditions, the smallest operational drop size for worst-case drift, as well as worst-case but unlikely wind conditions. While these assumptions may have suited the purposes for determining buffer zones, the assumptions are not ideal for many situations. When it comes to controlling drift, Hewitt (2000) stated that since the majority of drift came from the swath that was the most downwind, slight adjustments to the swath were in reducing drift. La Mer and Hochberg (1948), however, suggested that selecting favorable meteorological conditions and carefully controlling droplet size will give the most control over deposition.

Payne et al. also looked into mortality rates in fish, but did not look into the mortality of food supplies for the fish. They did, however, state that it would be wise to keep mortality levels of the food supply below the levels that would result in fish mortality. Schleier et al. (2008) found that the risk to aquatic life from pyrethrins and PBO, which are active ingredients in several insecticides used in the study, was minimal when applied aerially. They did not take into account the synergistic effects of pyrethrins from sources other than aerial. This study also did not mention the potential effects these products would have on food sources, and if an impact of this nature would indirectly affect the vertebrates. Because of these factors, the prevention of drift into waterways is still a point of concern.

The goal of this research was to provide recommendations for buffer zones to prevent contamination of adjacent water bodies and honey bee hives through field-measured deposition data and associated AGDISP<sup>®</sup> modeling over a range of distances.



## 4.2 Materials and Methods

AGDISP<sup>®</sup> (version 8.24, USDA Forest Service) was used to model deposition of five public health insecticides into waterways and at terrestrial points at various wind speeds and distances from the application. The model was first calibrated to field data obtained in a joint study with East Baton Rouge Mosquito Abatement and Rodent Control and Louisiana State University. AGDISP<sup>®</sup> is primarily an aerial application drift model that recently incorporated a mechanistic ground boom sprayer drift model that is intended only for flat fan and air-induction type spray nozzles (Teske et al. 2009). It has been extensively validated in these uses, and Thistle et al. (2005) give a detailed description of the mathematics that run the model (Hewitt 2008, Duan et al. 1992). Because of the absence of alternative spray drift models for use with truck-mounted, air-assisted public health sprayers, a number of assumptions, discussed in the Modeling Inputs section, were made in an effort to extrapolate buffer zone estimates based on matching modeled predictions to field measured data.

### 4.2.1 Field Setup

Field trials were conducted with a truck-mounted, air-assisted sprayer (Curtis Dyna-Fog, Ltd. Model LV-8). This configuration, while common for mosquito control operations, is not used in agricultural spraying. The test field was 27 m by 91 m (90 ft by 300 ft) with three rows of deposition sites set 9.1 m (30 ft) apart configured in six columns. The space between each column was 15 m (50 ft) and the first column was 15 m (50 ft) from the spray swath. The field was oriented with the wind blowing across the field from the spray line, as seen in Figure 4.1. Each deposition site included a stand holding one vertical circular cage each of 15 mosquitos and 15 honey bees to measure mortality and one impinger (spinner) (Leading Edge Associates, Waynesville, NC). The spinners used two 25mm uncoated glass microscope slides set 16cm apart (outside edge to outside edge), and the slides rotated at a velocity of 5.6m/s to measure the deposition concentration of each chemical at each site. Each chemical was applied in three replications and was mixed with Tinopal<sup>®</sup> optical brightener (BASF, Research Triangle Park, N.C.) at a concentration of 5g/L for a total of 15 test runs (5 pesticides x 3 replicates). Meteorological data (wind speed and direction) were available from the National Climatic Data Center (NCDC 2014). For each spray, the insects were counted for mortality. The exposed slides were washed with 10mL per sample of water plus 10% v/v isopropyl alcohol with 5 ml of the

final wash solution decanted in the borosilicate vials. The vials were processed for fluorescence using a spectrofluorophotometer (Model RF5000U, Shimadzu, Kyoto, Japan) with an excitation wavelength of 372nm, an emission at 427nm, and a minimum detection level of 0.00007mg/cm<sup>2</sup> for Tinopal®. The measured fluorescence values were converted to volume of spray solution using pre-mixed standard solutions of known dye concentration. These values were further converted to a concentration of insecticide using the solution mixing rates from the field study. Corrections were then made for the collection efficiency of the spinners and the results converted to volume of active spray per area sampled, as detailed by Fritz, et al. (2011), these values were used to validate the modeling results.

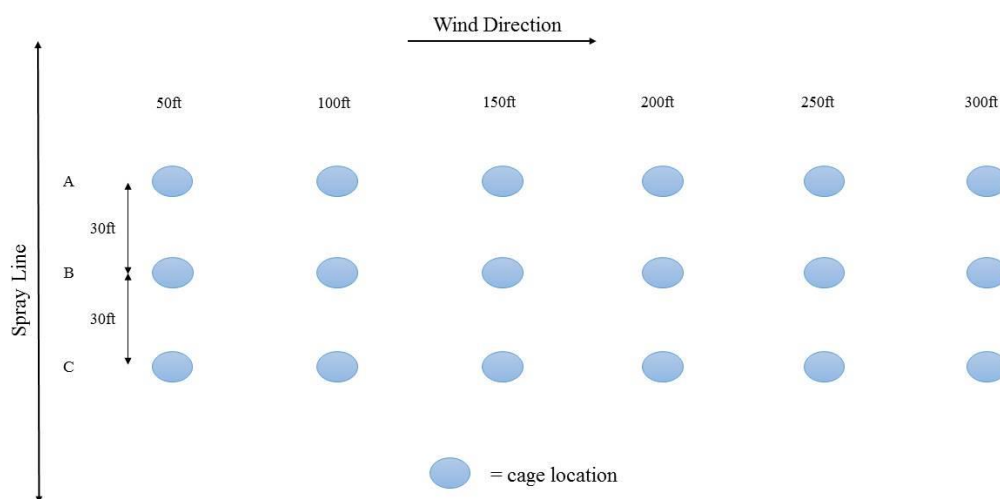


Figure 4.1. The field configuration used in calibration data collection in Baton Rouge, Louisiana June 2-6 and October 21-24, 2014.

#### 4.2.2 Modeling Inputs

Using the meteorological conditions monitored at the time of the spray trials, AGDISP® input values were established. A wind speed of 1.8 m/s (4 mph), a temperature of 18° C, and a relative humidity of 50% were used. Atmospheric stability was assumed to be the default setting of

“overcast.” The surface of the spray area was flat with no canopy. As mentioned previously, AGDISP® is not designed to model the truck mounted, air-assist sprayer used as part of this study; however, the inputs used for the ground sprayer model with AGDISP® can be modified to fit the operational parameters of the sprayer used in the study. A number of engineering judgments were required to determine these appropriate input conditions. The application method was set to “Ground,” with the nozzle type selected as “Flat Fan” with a boom pressure of 414 kPa (60 psig) and a single spray line. The effective swath width was set at 15 m (50 ft) based on the operational practices of the mosquito control district. Beyond these fairly basic inputs, three other critical inputs had to be determined to represent most appropriately the conditions in the field within the confines of the available modeling inputs. These include the Application Technique (which incorporates number of nozzles and spray droplet size distribution), Spray Material, and Release Height of the spray.

The Application Technique settings were modified from default such that the spray boom was set to a single nozzle, centered on the boom. The droplet size distribution used the “Parametric” option under the “User-defined” drop distribution type. The DV0.5 was entered as 55 µm with a relative span of RS=1.5. These values were based on previous evaluations of the LV-8 sprayer conducted using a water and non-ionic solution (Hoffmann et al. 2010). The DV0.5 values for the LV-8 sprayers tested by Hoffmann et al. ranged from 40 to 80 µm with a mean of 58 µm. Previous sprayers tested with both BVA and water with non-ionic surfactant tend to show BVA DV0.5 values that are approximately 5% less than the water solution, all other settings remaining the same.

The Spray Material settings within AGDISP® were used to establish the fraction of the spray material that is volatile and evaporates during the course of airborne transport. This results in changes in both droplet size and concentration of spray material with increasing transport distances. The spray solutions in this project were all oil-based and were further diluted into an oil carrier (BVA 13, a light petroleum oil used as a carrier for public health and agricultural sprays), meaning very little to no evaporation occurred. To aid in comparison with field data for the initial modeling efforts, the percent of tank mixture set as “Active” was 100%; meaning that the AGDISP® results for deposition that are reported as mass of active ingredient per area actually represent the mass of the total spray solution (carrier oil plus active product) per area. The last critical input within the Spray Material settings was the spray volume rate. The spray

plot established for the application field trials was 0.97 ha with each spray pass applying 239 mL within the plot. This corresponds to 246 mL/ha, which equals 0.026 gal/ac (AGDISP<sup>®</sup> input units).

The release height of the spray plume was the final critical input. While the spray nozzle exit was at approximately 1.8 m (6 ft), the air-assisted sprayer propelled the spray cloud to an estimated 3.7 - 4.6 m (12-15 ft) height prior to coming to ambient wind conditions. A series of modeling runs were conducted adjusting the spray release height from 3.7 to 4.6 m, with the 3.7 m resulting in the closest fit to the field measured data.

#### 4.2.3 Deposition Assessment

To determine properly how buffer zone distance based on aquatic and honey bee health affects dosage levels, actual spray solution mixture rates of the active ingredients were needed. Each product tested as part of this study was applied at both a high and low rate, as specified on the label of each product. AGDISP<sup>®</sup> was used to model the active fraction deposition and concentrations at terrestrial and aquatic locations as well as concentrations of material aloft at specified distances, assuming the active fraction of the spray solution is known. For this study, the active ingredient application rates were converted to active fraction rates with the remainder of the solution being the carrier fraction (Table 4.1). These values were directly input into AGDISP<sup>®</sup> under the Spray Material inputs with 100% of both the active and carrier being specified as nonvolatile (Figure 4.2). As mentioned before, the spray volume rate was 0.026 gal/ac, and was the same for each trial. The rate was varied by changing the amount of active ingredient (a.i.) in the carrier.

Table 4.1. Insecticides and rates used with active and carrier fractions of the spray solution.

Insecticide	Active Ingredient Application Rate (g/ha)	Active Fraction	Carrier Fraction
Aqua-Pursuit™	High – 7.9	0.03	0.97
	Low – 1.7	0.01	0.99
Duet®	High – 8.9	0.03	0.97
	Low – 3.0	0.01	0.99
Fyfanon®	High – 67.3	0.24	0.76
	Low – 33.7	0.12	0.88
Scourge®	High – 7.9	0.03	0.97
	Low – 1.7	0.01	0.99
Zenivex®	High – 7.9	0.03	0.97
	Low – 2.0	0.01	0.99

**Spray Material**

**Properties**  
 Name: BVA 13  
☐ Spray Material Evaporates  
 Spray Volume Rate: 0.026 gal/ac

**Fractions**  
 Active Fraction: 0.03  
 Nonvol. Fraction: 1

**Tank Mix**

**Active Solution**  
 % of Tank Mix: 3  
 Fraction of Active Solution that is nonvolatile: 1

**Additive Solution(s)**  
 % of Tank Mix: 97  
 Fraction of Additive Solution(s) that is nonvolatile: 1

**Carrier**  
 % of Tank Mix: -2.79E-06

**Total**  
 % of Tank Mix: 100

**Calculation Control**  
 Enter ☐ Fractions ☒ Tank Mix

**Pie Chart Legend:**  
 Nonvolatile Active (3 %)  
 Nonvolatile Additive(s) (97 %)  
 Other Nonvolatiles (-2.79E-06 %)

Figure 4.2. AGDISP® Spray Material input was specified as 100% nonvolatile.

With all inputs specified, the model was run and the results processed. Deposition assessments were performed for water bodies for each spray application to determine the required buffers such that deposition into a water body was less than lethal based on specified toxicity levels for each product applied. For the products used in this study, there were ranges in the published toxicity data, depending on the species of concern and the study done. Rather than try to cover full ranges for all species, a sampling of ranges is given in Table 4.2. Ideally, determining buffer zones would depend on a specific species of concern. AGDISP<sup>®</sup> allows for a direct calculation of distance and/or concentration for aquatic areas, terrestrial points and terrestrial areas. For aquatic areas, the water body width and average depth can be defined. However, the default values of 208.7 m (684.7 ft) width and 6.559 m (21.52 ft) depth are prescribed for worst-case assessments by the USEPA and were used for this work.

Table 4.2. Aquatic toxicity values used for the deposition assessment.

Product	Aquatic Toxicity Values ( $\mu\text{g a.i./L}$ )	Reference
Aqua-Pursuit <sup>™</sup>	1.8 - 2.5	Precision Control Technology, Inc. 2011
Duet <sup>®</sup>	0.154 - 19.55	Key et al. 2011
Fyfanon <sup>®</sup>	1 – 200	Cheminova Inc. 2002
Scourge <sup>®</sup>	1.25 - 100	Bayer Environmental Science 2013
Zenivex <sup>®</sup>	3.3 – 8.5	Wellmark International 2010

The previous work in Chapter III was used to define the airborne toxicity levels for each of the products used to determine the buffer distance to prevent honey bee lethal toxicity. The previous work used dilutions of the active products that were applied into a controlled bioassay wind tunnel containing caged bees.

For each solution sprayed, the previous paper has LC<sub>50</sub> and LC<sub>10</sub> values in the form of  $\mu\text{g}$  of active ingredient per mL of non-active and dilution material. It was necessary to make the conversion to an estimated airborne density in  $\mu\text{g}/\text{cm}^3$  that would be presented to the honey bees.

The LC<sub>50</sub> and LC<sub>10</sub> values in µg/mL were multiplied by the 0.5 mL spray volume used, then divided by the tunnel's area (15 cm diameter or 176.6 cm<sup>2</sup>) (WHO 2009). The LC<sub>50</sub> and LC<sub>10</sub> values for each product as well as the estimated airborne density are shown in Table 4.3.

Table 4.3. Conversion of LC<sub>50</sub> and LC<sub>10</sub> values from µg/mL to µg/cm<sup>2</sup>.

Product	LC <sub>10</sub> (µg a.i./mL inactive dilutant)	LC <sub>50</sub> (µg a.i./mL inactive dilutant)	Estimated Airborne Density (µg/cm <sup>2</sup> )	
			LC <sub>10</sub>	LC <sub>50</sub>
Aqua-Pursuit™	1350	2390	3.8	6.8
Duet®	395	2190	1.1	6.2
Fyfanon®	516	2430	1.5	6.9
Scourge®	129	351	0.4	0.99
Zenivex®	691	1370	2.0	3.9

## 4.3 Results and Discussion

### 4.3.1 Validation of Model

Model and field data are shown in Table 4.4. Both model and field data are representative of the total spray volume (carrier plus active). As mentioned previously, the spray volume rate was the same for each trial, and the rate was varied by changing the amount of active ingredient in the carrier. Because of this, these values that used the total spray volume should all be similar.

Because these results are being used to validate the model, total spray volume is used for ease of comparison. The transport aloft values from the model were quite close to the measured values in the field and were considered a good fit for low wind speeds, similar oil based pesticides, and the model of ground sprayer used. Weather in the field could not be controlled, while the atmospheric variables remained constant in the model. Consequently, a much greater difference in model and field data was expected. The values are decreasing in both data sets, with the exception of the model values from 15 to 30 m (50 to 100 ft). This can be explained by the

“perfect” atmospheric conditions in the model, where the release height of 3.7 m (12 ft) would take more than 15 m (50 ft) of horizontal transport for the majority of the droplet cloud to come down vertically to 1.8 m (6 ft), and the concentration at 1.8 m (6 ft) would peak somewhere after 15 m (50 ft), then decrease (Table 4.4). The field data, averaged over multiple repetitions, generally show a steady and pronounced decrease in 1.8 m transport concentrations from 15 to 30 m. No field data was obtained for Zenivex<sup>®</sup>.

Table 4.4. A comparison of the 1.8 m transport aloft values from AGDISP<sup>®</sup> and the values corrected for collection efficiency from the field study for both high and low application rates.

Distance (m)	Model ( $\mu\text{g}/\text{cm}^2$ )	Field ( $\mu\text{g}/\text{cm}^2$ )							
		Aqua-Pursuit <sup>™</sup>		Duet <sup>®</sup>		Fyfanon <sup>®</sup>		Scourge <sup>®</sup>	
		Low	High	Low	High	Low	High	Low	High
15	6.8	9.0	6.5	4.2	33	17	16	2.2	2.7
30	6.8	7.0	4.7	1.9	15	19	5.7	1.4	2.6
46	6.7	4.3	3.1	0.75	5.1	7.5	7.3	1.5	2.2
61	6.5	3.5	2.2	0.67	4.2	4.8	4.6	1.0	2.2
76	6.3	2.5	2.1	0.45	2.7	3.9	1.7	0.86	2.1
91	5.9	2.4	2.0	0.50	2.6	2.2	1.6	1.5	2.0

#### 4.3.2 Deposition Assessment

To determine water body spray deposition at both the high and low application rates, AGDISP<sup>®</sup> was run from 0 to 61 m (200 ft) (Tables 4.5, 4.6). Given the aquatic toxicity values presented in Table 4.2, these spray rates do not produce concentrations at levels of concern. The model only takes into account this spray, however, not any runoff or drift from other sources.



Table 4.5. Model generated concentrations at high application rates.

Distance (m)	Aqua-Pursuit™	Concentration (µg a.i./L)			
		Duet®	Fyfanon®	Scourge®	Zenivex®
0.00	4.49E-03	4.49E-03	3.59E-02	4.49E-03	4.49E-03
15.2	4.42E-03	4.42E-03	3.54E-02	4.42E-03	4.42E-03
30.5	4.20E-03	4.20E-03	3.36E-02	4.20E-03	4.20E-03
61.0	3.67E-03	3.67E-03	2.94E-02	3.67E-03	3.67E-03

Table 4.6. Model generated concentrations at low application rates.

Distance (m)	Aqua-Pursuit™	Concentration (µg a.i./L)			
		Duet®	Fyfanon®	Scourge®	Zenivex®
0.00	1.50E-03	1.50E-03	1.80E-02	1.50E-03	1.50E-03
15.2	1.47E-03	1.47E-03	1.77E-02	1.47E-03	1.47E-03
30.5	1.40E-03	1.40E-03	1.68E-02	1.40E-03	1.40E-03
61.0	1.22E-03	1.22E-03	1.47E-02	1.22E-03	1.22E-03

AGDISP® was run to determine the transport aloft at downwind distances out to a maximum of 61 m (200 ft) to estimate the buffer distance needed to reduce airborne spray concentrations to that below the LC<sub>50</sub> values for honey bees for each product. AGDISP® is only recommended out to 305 m (1000 ft), so another model would need to be used for further distances. The maximum airborne density at each distance was used, regardless of vertical height. For each distance of interest, the distance was entered into AGDISP® under Transport. The model was run, and the maximum airborne density determined from the Transport Aloft data (Table 4.7). None of the products used reached the estimated airborne density that would cause 50% mortality (Table 4.3). In fact, all but Fyfanon® were below the LC<sub>10</sub> values for honey bees.

Table 4.7. Maximum transport aloft ( $\mu\text{g a.i./cm}^2$ ) values up to 61 m.

Distance (m)	Height (m)	Transport Aloft ( $\mu\text{g a.i./cm}^2$ )									
		Aqua-Pursuit™		Duet®		Fyfanon®		Scourge®		Zenivex®	
		High	Low	High	Low	High	Low	High	Low	High	Low
0.30	3.3	0.33	0.11	0.33	0.11	2.6	1.3	0.33	0.11	0.33	0.11
3.0	3.2	0.31	0.10	0.31	0.10	2.4	1.2	0.31	0.10	0.31	0.10
15	3.0	0.25	0.085	0.25	0.085	2.0	1.0	0.25	0.085	0.25	0.085
30	2.8	0.23	0.075	0.23	0.075	1.8	0.90	0.23	0.075	0.23	0.075
61	2.4	0.20	0.066	0.20	0.066	1.6	0.80	0.20	0.066	0.20	0.066

#### 4.4 Conclusions

The AGDISP® model provided a good estimate of field data for a ground-mounted flat sprayer when the correct parameter values were selected. For the mosquito control products tested there was little need for buffer zones to protect honey bees or water bodies. The only exception was Fyfanon®, which exceeded the  $\text{LC}_{10}$  values for honey bees at 61 m from the application point when applied at the high application rate. There are obviously limits to this study, but one could use the validation of the AGDISP® model for this type of application. Then altering the model for different pesticides, wind speeds, or foggers could be relatively straight forward. It should be noted that the analysis presented is an approximation. If a product is suspected to be deadly in small doses to aquatic life or honey bees it should be rigorously field tested and a more conservative approach should be taken before application.

## CHAPTER V

### CONCLUSIONS

It is difficult to summarize the findings of even a single group of pesticides into a simple recommendation, as seen in Druzina and Stegu (2007), Simo and Zhang (2002), and Amweg et al. (2006). This, combined with many potential entry points for pesticides, especially in urban areas where their use is less monitored and they are applied in greater amounts per unit area, leads to the suggestion that a potential focus might be on just how prevalent these pesticides are and what the actual concentrations are in natural systems. Often the concentrations achieved from a single application are not going to cause concerning mortality levels, but multiple, unrelated applications are what cause concern, as seen in Weston (2005). It is also very difficult to determine the sub-lethal chronic effects of exposure to pesticides and the unintended effects of multiple pesticides working together to cause mortality. There's much we do not know yet about the way these compounds act in complicated natural systems, and more studies will need to be performed involving specific compounds and their metabolites and specific areas of concern.

In Chapter III, it was found that the insecticides tested had negligible impact on honey bee mortality even at maximum usage. The results from this study will provide guidance to mosquito abatement district managers to properly select public health insecticides that cause minimum toxicity effects on honey bees. The results strengthen the notion that most public health sprays should be done at dusk or later in the day when honey bees are not active outside and remain inside their hives. It should be noted that this study did not examine the potential chronic effects of any of these insecticides at sub-lethal doses.

In Chapter IV, the AGDISP<sup>®</sup> model provided a good estimate of field data for a ground-mounted flat sprayer when the correct parameter values were selected. For the mosquito control products tested there was little need for buffer zones to protect honey bees or water bodies. The only exception was Fyfanon<sup>®</sup> (malathion), which exceeded the LC<sub>10</sub> values for honey bees at 61 m from the application point when applied at the high application rate. There are obviously limits to this study, but one could use the validation of the AGDISP<sup>®</sup> model for this type of application. Then altering the model for different pesticides, wind speeds, or foggers could be relatively

straight forward. It should be noted that the analysis presented is an approximation. If a product is suspected to be deadly in small doses to aquatic life or honey bees it should be rigorously field tested and a more conservative approach should be taken before application.

Preventing entrance to waterways and deposition onto honey bee hives should still be the main focus when applying these pesticides. This can be done by applying in prime meteorological conditions in the case of drift and volatilization and preventing runoff by using vegetated buffer zones in agricultural areas, avoiding the application to pavement and hard surfaces in urban areas in the case of runoff. As well as being aware of honey bee hive locations downwind of the spray swath.

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## APPENDIX A

## FIGURES

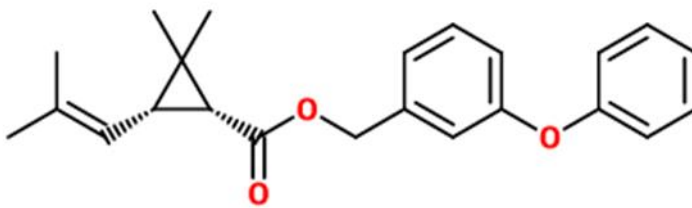


Figure A-1. Chemical structure of phenothrin (sumithrin).

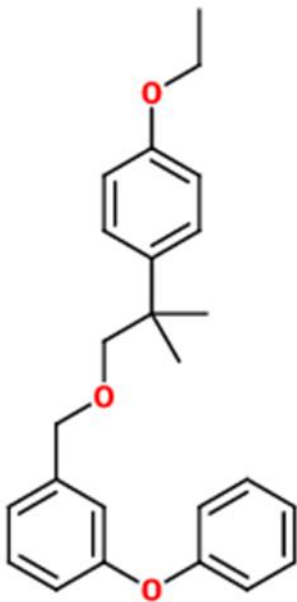


Figure A-2. Chemical structure of etofenprox.

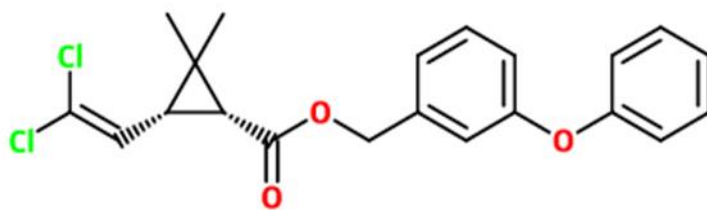


Figure A-3. Chemical structure of permethrin.

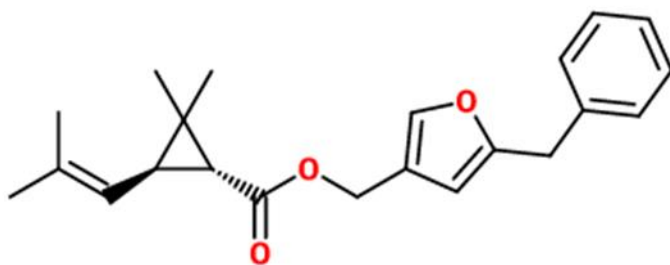


Figure A-4. Chemical structure of resmethrin.

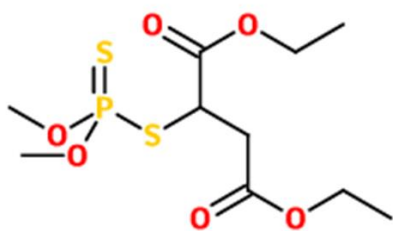


Figure A-5. Chemical structure of malathion.

## APPENDIX B

### TABLES

Table A-1. Water solubility of the selected compounds.

Compound	Solubility in Water (mg/L)	Reference
Malathion	148*	WHO 2003
Permethrin	0.00450	Vasquez et al. 2010
Sumithrin	2.00*	WHO 2004
Resmethrin	insoluble	Bayer Environmental Science 2013
Etofenprox	0.404	Vasquez et al. 2010

\*At 25°C

Table A-2. Henry's Law constants of the selected compounds.

Compound	Henry's Law Constant (Pa-m <sup>3</sup> /mol)	Reference
Malathion	4.96E-04	NIH 2012
Permethrin	2.07E-01	Vasquez et al. 2010
Sumithrin	6.89E-01	EPA 2008
Resmethrin	1.32E-02	NIH 2010
Etofenprox	3.49E-03	Vasquez et al. 2010